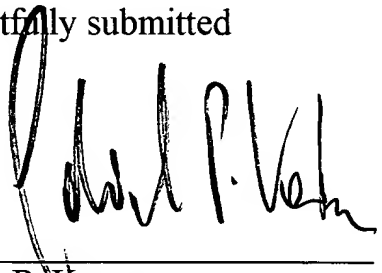
REMARKS

Claims 23-45 are in the application.

Also enclosed herewith is a redlined copy of the substitute disclosure, showing the changes that were made in the original translation. No new matter was added.

Favorable consideration of the claims is respectfully urged.

Respectfully submitted



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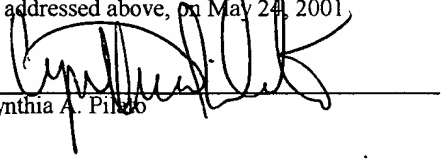

Cynthia A. Pinto

~~0107-031 Anti-petasin Antibodies, Methods for Making and Therapeutic Process~~

~~Field of invention~~

# -ANTI-PETASIN ANTIBODIES, METHODS FOR THE PRODUCTION THEREOF AND THEIR USE

~~Description~~

The invention relates to anti-petasin antibodies for detecting petasin or petasin protein conjugates in physiological liquids which do not show any cross reactivity to derivatives, structural analogues or metabolics of petasin, methods for producing them by means of immunization by petasin derivatives which are ~~suitably~~preferably coupled to a carrier molecule, and to their use and a test kit.

~~Background~~

Petasin, a component of butterbur extracts is ~~a~~ as is known – an ester consisting of petasol and angelic acid which already for a longer time has been used as vegetable spasmoanalgesic for combatting spasms of the gastrointestinal tract, in particular ureteral colics, spastic bronchititis and migraine and ~~also as an antiphlogistic~~ antiphlogistically (B. Debrunner et al.; Pharm. Acta Helv. 72, 359-380 (1998). In addition, an antitumour effect is ~~attributedascribed~~ to petasin drugs (B. Meier et al., Hagers Handbuch der pharmazeutischen Praxis (Manual of pharmaceutical practice), 5th edition, p. 81-105, Springer-Verlag (1994)). ~~Also~~In the mean time also latest findings relating to the effects on the biosynthesis of leukotrienes are available (D. Pichl et al., Planta Medica, 60, 318-322 (1994)).

After ~~oral~~peroral application of petasin drugs only concentrations in the range of a few ng/m~~l~~l are to be expected in body fluids of healthy ~~subjects~~probands. ~~Due~~Owing to this background biological, physical and chemical methods of detection applied ~~in~~for characterizing the drug itself ~~cannot~~may not be used for quantifying petasin in body fluids. Even most up-to-date analytical methods such

as the HPLC usually applied are not sufficiently sensitive or not ~~suitable~~ ~~due~~suited owing to ~~their large time requirement~~ requiring much time for large numbers of samples.

~~Brief description of the invention~~

~~It is for that reason that the object of the present invention is~~

That is why the invention was based on the task to provide methods of detecting petasin, in particular suitable methods with a high sensitivity and specificity which allow a good bioavailability for the desired pharmacokinetic investigations.

~~The~~

According to the present invention immunochemical methods of detection ~~according to the present invention~~ meet the requirements for sensitivity and specificity thus not requiring an additional extraction or concentration of the sample ~~to advance~~advancing the proper determination as it is required when applying chromatographic methods ~~of the prior art~~.

---

It was possible to accomplish this task by providing ~~an~~ anti-petasin ~~antibody for detecting petasin or petasin protein conjugates in physiological fluids wherein the antibody does~~antibodies which, in particular, do not show any cross reactivity to derivatives, structural analogues or metabolites of petasin.

---

The antibodies according to the present invention are produced ~~by preparing polyclonal or monoclonal antibodies by mammals and/or birds~~ with petasin~~the aid or a~~f derivative ~~thereof~~s of petasin, which are ~~suitably~~preferably coupled to a carrier molecule. ~~It was found, t~~To our surprise, ~~that thus~~ it was thus possible to avoid a production of antibodies directed against the coupling group of petasin or a potentially occurring modification of the immunodominant epitope situated in the vicinity of position 8.

~~Brief description of the drawing~~

~~The invention is described in greater detail below, with reference being had to the sole figure of the drawing, showing the mean petasin concentration changes of petasin in serum, as a function of time.~~

## Detailed description

The polyclonal or monoclonal antibodies are produced by immunization of mammals and/or birds by petasin or petasin derivatives of the general formula (I)



(I)

and obtained by means of the hybridomae techniques or recombinantly with the aid of antibody libraries.

The

Preferably the following derivatives coupled to a carrier molecule are suitably used:

(a)

Derivatives of petasin of the general formula I where the keto group in position 8 is

replaced by a carboxyl group and coupled to a bovine serum albumin by means of EDAC;

~~(b)~~ Derivatives of petasin of the general formula I where the keto group in position 8 is replaced by a carboxyl group and coupled to a bovine serum albumin or fibrinogen through an activated hydrazide dextran with the carboxyl group being ~~suitably~~ preferably inserted with carboxymethylhydroxyamine forming oxime;

~~(c)~~

Derivatives of petasin of the general formula I where the double bond in positions 11,12 is brominated and coupled to bovine serum albumin activated by means of a Traut's reagent; ~~and~~.

~~(d)~~ Derivatives of petasin of the general formula I where angelic acid has been split off and the remaining petasol has been coupled to a carrier through chloroformic acid ester.

---

The anti-petasin antibodies thus produced do not show any ~~crossreactivity~~cross reactivity to derivatives, structural analogues or metabolites of petasin and are used for detecting petasin or petasin-protein conjugates in physiological liquids with either petasin, petasin protein conjugates or the anti-petasin antibodies ~~suitably showing a marker, such as enzymes~~showing preferably a marker. The reactants are preferably available in a homogeneous solution.

Enzymes, fluorescent dyes, radioisotopes or redoxactive compounds~~. The reactants are suitably available in a homogeneous solution~~used as markers.

---

Petasin bound to antibodies is optically, electrochemically, fluorimetrically or radiochemically detected, ~~suitably~~preferably optically by means of colour reagents or ~~by chromatography~~chromatographically.

---

In one ~~embodiment of the present invention~~variant either anti-petasin antibodies, the petasin to be detected, or the petasin protein conjugates are bound ~~onto~~to a solid phase with a washing process taking place between the reaction steps.

---

~~The~~

If necessary, the solid phase is ~~suitably~~ chemically activated, ~~wherein adsorptive or covalent bonding takes place~~with binding of the anti-petasin antibodies, ~~or~~ the petasin to be ~~determined~~,detected or the petasin-protein ~~conjugates~~conjugate to it being effected adsorptively or covalently. Polystyrene is ~~suitably~~preferably used as solid phase.

---

In addition, the solid phase ~~can~~may have a differing geometric shape, thus e.g. the shape of a microtitration plate, a tube or ~~have~~ a spherical or ~~plane~~planiform shape.

---

~~The~~

Furthermore, the invention ~~furthermore~~ relates to a test kit for detecting petasin in physiological liquids comprising
anti-petasin antibodies,
a solid phase, ~~such as~~ of polystyrene,
washing solution,
dilution buffer,

marked petasin or a marked anti-species antibody,
a marker-specific detection system, ~~suitably~~preferably an enzyme substrate.

~~The~~

~~Hereinafter~~ the invention is ~~hereinafter~~ explained in greater detail by ~~reference to the following~~means of examples.

**Examples**

~~A)~~

Production of immunogenes

**Petasin oxime:**

10 mg ($3.3 \times 10^{-5}$ mol) of petasin are to be dissolved in 5 ml of ethanol, 15 mg ($6.8 \times 10^{-5}$ mol) of carboxymethoxylamine hemihydrochloride (Sigma-Aldrich) are to be added and 5 M sodium hydroxide solution are to be added drop by drop until a pH of 12 is will be reached. The batch is refluxed for 4 hours h, evaporated to dryness on a water bath, washed with 2 M hydrochloric acid and dissolved in a mixture of 1 ml of dioxane and 2 ml of DMSO and stored at -70 °C.

Thin-layer chromatography: $R_f$ value (silica gel G60, chloroform) = 0.42 (petasin: 0.16).

Oxime is formed as sole reaction product.

**Petasin oxime bovine serum albumin:**

32 mg ($4.8 \times 10^{-7}$ mol) of bovine serum albumin (BSA) are to be dissolved in 4 ml of

PBS (solution A).

7 mg ($1.8x10^{-5}$ mol) of petasin oxime, dissolved in 1 ml of dioxane/DMSO = 1:2 (v/v), 16 mg of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDAC) are to be added and while stirringbeing stirred incubated for 30 min. at room temperature (solution B).

Solution B is added dropwisedrop by drop to solution A, stirred for 6 hoursh at room temperature, subsequently dialyzsed at 4°C against 3x0.5 l of PBS (0.01 M phosphate, 0.15 M NaCl, pH = 7.4) and stored at –70°C.

**Petasin-dextran proteins:**

7 mg ($1.8x10^{-5}$ mol) of petasin oxime, dissolved in 1 ml of dioxan/DMSO = 1:2 (v/v) are added dropwisedrop by drop to 32 mg of bovine serum albumin ($4.8x10^{-7}$ mol) or fibrinogen in 4 ml of PBS, and 0.5 mg ($1.5x10^{-4}$ mol hydazide groups) of activated hydrazide dextran (Pierce, Code 20900) are to be added. Thereupon, 16 mg of 1-ethyl-3-(3-dimethylaminopropyl(carbodiimide (EDAC) are to be added and the mixture is to be incubated for 4 hoursh at room temperature. Thereupon, a dialysis is carried out at 4°C against 3x0.5 l of PBS (0.01 M phosphate, 0.15 M NaCl, pH = 7.4). Storage is effected at –70°C.

**Bromopetasin bovine serum albumin:**

*Brominating of petasin:*

10 mg ($3.1 x10^{-5}$ mol) of bromine in 1 ml of dichloromethane, dissolved in 3 ml of

dichloromethane, are added drop by drop with swirling to 5 mg of ( $3.3x10^{-5}$ mol) petasin. Thereupon, the batch is evaporated to dryness on a water bath and taken up in 1 ml of DMSO.

Thin-layer chromatography: $R_f$ value (silica gel G60, chloroform) = 0.51 (petasin: 0.16).

*Thiolation of bovine serum albumin*:

40 mg ($6x10^{-7}$ mol) of bovine serum albumin is to be dissolved in 1 ml 0.1 M of phosphate buffer, pH = 8.0, and 20 mg ($1.4x10^{-4}$ mol) of 2-iminothiolane hydrochloride (Traut's reagent) are to be added and incubated for 40 min. at room temperature. Subsequently, with the aid of a column filled with Sephadex G25 (1x10 cm) an buffer exchange is carried out against 0.1 M phosphate buffer at pH = 7.2. 4 mg ($8.4x10^{-6}$ mol) of bromopetasin are added while stirringbeing stirred for dissolving the thiolated protein and an incubation is effected for 3 hoursh at room temperature, thereupon a dialysis is carried out at 4°C against 3x0.5 l of PBS (0.01 M phosphate, 0.15 M NaCl, pH = 7.4).

**Petasol bovine serum albumin:**

0.7 mg ($2.9x10^{-6}$ mol) of petasol are dissolved in 200 µlµl of dried dioxane/DMF = 1:1 (v/v), 2 mg ($7.9x10^{-6}$ mol) of 5-norbornene-2,3-dicarboximidyl chloroformic acid ester and 4 mg ($3.3x10^{-5}$ mol) of 4-dimethyl amino pyridine are added and the mixture is incubated for 1 hourh at room temperature excluding atmospheric humidity. Thereupon, this solution is added dropwisedrop by drop with stirring to 10 mg ($1.5x10^{-7}$ mol) of bovine serum albumin, dissolved in 0.5 ml of PBS and

incubated for 2 hours at room temperature. Thereupon, it is dialyzed at 4°C against 3x0.5 l of PBS (0.01 M phosphate, 0.15 M NaCl, pH = 7.4) and protein conjugate is stored at –70°C.

~~B)~~

Production of anti-serum

Immunization is administered effected in rabbits as primary subcutaneous injection by subcutane and intramuscular injection with always 3 mg of petasin-BSA in a complete Freund's adjuvant. The secondary injection is effected four weeks after the primary injection. After further two weeks the first booster injection is ~~administered~~effected, a second one is carried out twelve weeks after the beginning of immunization, in an incomplete Freund's adjuvant. About eight weeks after starting immunization at the first blood sample is taken which is supplemented by a further one after four weeks. Exsanguination is carried out after 16 weeks.

The antiserums obtained are subjected to a titer determination for specific anti-petasin antibodies by means of an enzyme immunoassay where petasin ovalbumin is bound to the surface of microtitration plates. The antiserums to be examined and the normal sera of the rabbits are subsequently incubated in a dilution series with the immobilized petasin. The bound antibodies are detected by incubation with a goat-anti-rabbit immunoglobuline enzyme conjugate (peroxidase) and subsequent visually evaluable substrate reaction.

~~C)~~

Enzyme immunoassay

**Petasin ovalbumin:**

4 mg of EDAC are added to 0.3 mg ($8x10^{-7}$ mol) of petasin oxime, dissolved in 100 µl of dioxane/DMSO = 1:2 (v/v) and incubated for 30. min. at room temperature. Subsequently, the batch is put into a solution of 5.5 mg ($1.2x10^{-7}$ mol) of ovalbumin in 3 ml of PBS, incubated for 2 hours at room temperature while being stirred and subsequently for 16 hours at 4°C. The reaction mixture is dialysed at 4°C against 3x0.5 l of aqua bidest. and the protein conjugate is stored at –70°C.

**Coating:**

Petasin ovalbumin is adsorptively bound to polystyrene microtitration plates in a concentration of 5 mg/l in 0.1 M carbonate buffer, pH = 9.5, (100 µl/well) for 16 hours at 4 °C and thereupon sucked off. After washing it two times with 300 µl/well washing buffer (PBS, 0.1 % Tween 20) it is blocked for 2 hours at room temperature with 150 µl/well blocking solution (0.6 % gelatine, 0.02 % sodium acid in PBS) and subsequently washed three times with washing buffer.

**Execution of the test:**

50 µl of the serum sample to be tested or the respective standard (1:4 dilution in a sample buffer (PBS, 1 % BSA, 0.1 % Tween 20, 0.01 % thiomersal) and 50 µl of an optimized anti-serum dilution in a sample buffer are simultaneously incubated with shaking for 1 hour at room temperature. Subsequently, the microtitration plate is washed three times with 300 µl/well/well of washing buffer and incubated for 30 min. at room temperature with 100 µl of anti-rabbit immunoglobulin peroxidase conjugate, diluted in sample buffer, and once more washed as above. Thereupon, it is incubated for 10 min. with 100 µl of a substrate solution ready for use (3.3', 5.5'-tetramethyl benzidine) per well and the reaction is stopped by adding 100 µl/well/well of 0.5 M sulfuric acid. The evaluation is carried out at 450 nm in a microtitration plate reader.

**Description of indication**

Plant extracts obtained by means of special methods from leaves or rhizomes of *Petasites hybridus L.* can may inhibit the 5-lipoxygenase. Thus, the arachidonic acid cascade is effectively interrupted in the case of allergic inflammations. In particular, the release of leukotriene from endogenic cells stimulated in the case of inflammations is stopped, inter alia also from eosinophilic and neutrophilic leukocytes.

Thus, such plant extracts are potential candidates for the therapeutic use in the case allergic inflammations such as allergic rhinitis, asthma, atopic dermatitis, colitis ulcerosa etc. First clinical experience proves the therapeutic efficiency of this plant extract in the case of allergic rhinitis. A prophylactic use of the extract in the case of

selected forms of migrainemigrain gave also gave indications to its efficiency.

---

In addition to detecting the plasma level required for the efficiency for relevant components of the extract, e.g. petasin, the knowledge of the pharmacokinetics of such relevant components isare urgently required for a medical use of the plant extract. With the anti-petasin antibodies according to the present invention in an enzyme immunoassay a secure detection of petasin in the blood in the lower ng range is achieved. The results of the followinga pharmacokinetic examination enclosed proves impressively its usability.

---

In a 1st phase of the clinical test for determining the pharmacokinetic parameters of tablets containing butterbur extract was a single oral administrations of 2 or 4 tablets to 24 clinically healthy men at the age between 18 and 40 years were evaluated.

The open, cross-over test was chosen as method, single administration of each dose in a randomized order with an interval of at least 7 days between the administrations.

Efficiency:

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Model-independent pharmacokinetic parameters for petasin

Statistical methods:

ANOVA, $ANOVA_{log}$, Wilcoxon-Mann-Whitney test, Wilcoxon-sign order test

Summary – conclusions

**Results:**

Petasin serum concentration (ng/ml)

| | after administering 2 tablets | | | | | | after administering 4 tablets | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ~~Time~~ Zeit p.a.(h) | N | Mean | S.D. | Min. | Median | Max. | N | Mean | S.D. | Min. | Median | Max. |
| 0 | 0 | 0,0 | | 0,0 | | 0,0 | 0 | 0,0 | | 0,0 | | 0,0 |
| 0,25 | 9 | 2,8 | 1,8 | 1,1 | 2,2 | 5,5 | 13 | 4,2 | 3,7 | 1,0 | 3,2 | 14,9 |
| 0,5 | 20 | 7,6 | 5,8 | 1,5 | 5,9 | 23,3 | 21 | 21,2 | 24,9 | 1,3 | 13,7 | 96,2 |
| 0,75 | 20 | 11,9 | 5,7 | 4,0 | 11,0 | 23,8 | 19 | 28,7 | 22,5 | 2,5 | 23,0 | 91,8 |
| 1 | 20 | 15,6 | 7,2 | 4,0 | 14,7 | 29,3 | 20 | 36,6 | 23,0 | 7,8 | 38,1 | 100,0 |
| 1,~~17~~1 67 | 20 | 21,0 | 16,1 | 5,6 | 15,3 | 62,9 | 20 | 47,3 | 29,1 | 7,5 | 43,6 | 100,0 |
| 1,5 | 20 | 19,3 | 12,0 | 5,1 | 16,1 | 47,3 | 19 | 40,8 | 22,3 | 12,2 | 32,4 | 90,7 |
| 1,833 | 20 | 18,2 | 11,3 | 7,8 | 14,7 | 44,3 | 20 | 32,0 | 20,1 | 13,8 | 26,8 | 100,0 |
| 2,167 | 20 | 16,3 | 8,3 | 7,3 | 14,5 | 31,7 | 20 | 28,9 | 15,0 | 11,4 | 27,5 | 76,1 |
| 2,5 | 20 | 13,6 | 6,4 | 5,9 | 10,2 | 26,6 | 19 | 24,3 | 10,7 | 8,4 | 26,1 | 40,9 |
| 3 | 20 | 8,8 | 4,1 | 3,1 | 7,7 | 18,3 | 20 | 17,9 | 10,0 | 7,2 | 14,6 | 49,0 |
| 4 | 20 | 4,5 | 2,6 | 1,7 | 5,2 | 11,2 | 20 | 9,5 | 5,2 | 2,9 | 8,1 | 20,8 |
| 5 | 20 | 4,1 | 2,3 | 1,5 | 3,4 | 8,8 | 21 | 12,4 | 16,5 | 3,2 | 7,3 | 81,4 |
| 6 | 18 | 3,2 | 1,7 | 1,2 | 3,1 | 8,1 | 21 | 5,8 | 3,8 | 1,6 | 5,0 | 14,7 |
| 8 | 18 | 1,9 | 0,8 | 1,0 | 1,6 | 4,2 | 19 | 3,9 | 3,1 | 1,4 | 3,1 | 15,7 |
| 12 | 13 | 1,6 | 0,5 | 1,1 | 1,4 | 2,9 | 18 | 2,7 | 1,0 | 1,3 | 2,5 | 5,1 |
| 24 | 6 | 1,5 | 0,5 | 1,1 | 1,3 | 2,3 | 10 | 1,3 | 0,4 | 1,0 | 1,0 | 2,3 |

Values below the detection limit (1 ng/ml) are equated with 0.

**Model-independent pharmacokinetic parameters (± S.D.)**

| parameter/dosage | | 2 tablets | 4 tablets |
|---|---|---|---|
| $C_{max}$ (ng/ml) | | 25,5 | 58,1 |
| | SD | ±14,8 | ± 26,7 |
| $t_{max}$ (h) | | 1,616 | 1,614 |
| | SD | ±0,499 | ± 0,926 |
| $AUC_{0\text{-}t(last)}$ (ng/ml*h) | | 65,30 | 151,15 |
| | SD | ± 35,61 | ± 68,21 |
| $AUC_{0\text{-}\infty}$ (ng/ml*h) | | 79,68 | 168,22 |
| | SD | ± 42,27 | ± 73,43 |
| $AUC_{Rest}$ (%) | | 18,3 | 10,8 |
| | SD | ± 7,9 | ± 4,9 |
| $T_{1/2}$ (h) | | 7,155 | 7,618 |
| | SD | ± 4,611 | ± 3,338 |
| MRT (h) | | 7,32 | 6,74 |
| | SD | ± 3,74 | ± 2,47 |

**Security parameters:**

No significant and clinically relevant modifications of the haematological and clinical.chemical laboratory parameters

**Undesired events:**

Undesired events did not occur.

**Conclusions:**

The resorption takes quickly place depending on the dose.

Both dosages shall be regarded to be equal as to their bioavailability.

Mathematical-statistical evaluation

~~1.~~

Pharmacokinetic and statistical calculations

The serum levels of petasin measured were the basis of the evaluation.

~~2.~~

Model-independent pharmacokinetic parameters

The averages and standard deviations (SD) of the pharmacokinetic parameters ~~are shown~~have been summed up in Table 1.

| Parameter/dosage | 2 tablets | 4 tablets |
|---|---|---|
| $C_{max}$ (ng/ml) ± SD | 25,5 ±14,8 | 58,1 ± 26,7 |
| **$t_{max}$ (h) ± SD** | 1,616 ±0,499 | 1,614 ± 0,926 |
| **$AUC_{0\text{-}t(last)}$ (ng/ml*h) ± SD** | 65,30 ± 35,61 | 151,15 ± 68,21 |
| $AUC_{0\text{-}\infty}$ (ng/ml*h) ± SD | 79,68 ± 42,27 | 168,22 ± 73,43 |
| $AUC_{Rest}$ (%) ± SD | 18,3 ± 7,9 | 10,8 ± 4,9 |
| $t_{1/2}$ (h) ± SD | 7,155 ± 4,611 | 7,618 ± 3,338 |
| MRT (h) ± SD | 7,32 ± 3,74 | 6,74 ± 2,47 |

The dose-dependent parameters $C_{max}$ and AUC are nearly proportional to the dose,

the deviations of the averages of all other parameters are nearly identical considering the standard deviations that were determined.

---

The big standard deviations have to be regarded as an expression of interindividual differences, notably of the speed of resorption, distribution and metabolism of petasin. Thus, after administering the low dose a petasin serum level above the determination limit of the analyzing method has not been detected at no time.

---

The calculation of the relevant bioavailability (calculation of the dose-corrected quotient of the pharmacokinetic parameters with a 90 % confidence interval) of the dose of 4 tablets compared with a dose of 2 tablets of the test medication shows:

Table 2
Comparative bioavailability
Butterbur 4 tablets versus butterbur 2 tablets

| adoption of distributions | Statistical method | $C_{max}$ point estimation | T/R (%) Conf.interv. from ... to Point estimation | $AUC_{0-\infty}$ Punkt-Schätz. | T/R (%) Conf.interv. from... tos |
|---|---|---|---|---|---|
| normal distr | ANOVA (x-Over) | 113,5 | 91,9 ... 135,0 | 106,2 | 86,5 ... 126,0 |
| log-normal distrib. | ANOVA log (x-Over) | 114,9 | 94,7 ... 139,5 | 109,1 | 92,5 ... 128,6 |
| distribution-free | Wilcoxon-Mann-Whitney Test | 113,5 | 87,7 ... 141,8 | 101,0 | 87,5 ... 121,3 |
| | Wilcoxons sign-order-test | 111,4 | 93,4 ... 135,4 | 104,5 | 90,8 ... 122,3 |

In the framework of the limits between 70 and 142.9 % for $C_{max}$ and between 80 and 125 % for AUC usually accepted in bioavailability tests the availability of both dosages is to be regarded as equal.

Table 3

Groups stastistic of the petasin concentration (ng/ml) in serum after administering 4 tablets

| Time p.a. | N | Mean | S.D. | Min. | Median | Max. |
|---|---|---|---|---|---|---|
| 0 | 0 | <1 | | | <1 | |
| 0,25 | 13 | 4,2 | 3,7 | 1,0 | 3,2 | 14,9 |
| 0,5 | 21 | 21,2 | 24,9 | 1,3 | 13,7 | 96,2 |
| 0,75 | 19 | 28,7 | 22,5 | 2,5 | 23,0 | 91,8 |
| 1 | 20 | 36,6 | 23,0 | 7,8 | 38,1 | 100,0 |
| 1,167 | 20 | 47,3 | 29,1 | 7,5 | 43,6 | 100,0 |
| 1,5 | 19 | 40,8 | 22,3 | 12,2 | 32,4 | 90,7 |
| 1,833 | 20 | 32,0 | 20,1 | 13,8 | 26,8 | 100,0 |
| 2,167 | 20 | 28,9 | 15,0 | 11,4 | 27,5 | 76,1 |
| 2,5 | 19 | 24,3 | 10,7 | 8,4 | 26,1 | 40,9 |
| 3 | 20 | 17,9 | 10,0 | 7,2 | 14,6 | 49,0 |
| 4 | 20 | 9,5 | 5,2 | 2,9 | 8,1 | 20,8 |
| 5 | 21 | 12,4 | 16,5 | 3,2 | 7,3 | 81,4 |
| 6 | 21 | 5,8 | 3,8 | 1,6 | 5,0 | 14,7 |
| 8 | 19 | 3,9 | 3,1 | 1,4 | 3,1 | 15,7 |
| 12 | 18 | 2,7 | 1,0 | 1,3 | 2,5 | 5,1 |
| 24 | 10 | 1,3 | 0,4 | 1,0 | 1,0 | 2,3 |

Values below the detection limit (1 ng/ml) correspond to 0.

From the attached Figure there can be seen that the medium maximum petasin concentration ($C_{max}$) has nearly doubled after administering double the dose. The medium time of reaching the maximum serum level ($t_{max}$) remains constant.

Patent claims

Anti-petasin antibodies for detecting petasin or petasin protein conjugates in physiologic fluids which do not show any cross reactivity to derivatives, structural analogues or metabolites of petasin.

Method for producing anti-petasin antibodies wherein polyclonal or monoclonal antibodies are produced by immunization of mammals and/or birds with petasin or petasin derivatives of the general formula I

and antibodies are obtained by means of the hybridome technique or recombinantly with the aid of antibody libraries.

Method according to claim 2 wherein derivatives coupled to carrier molecules are used as petasin derivatives for immunization.

Method according to claim 3 wherein derivatives of petasin are used for immunization where the keto group in position 8 has been replaced by a carboxyl group and coupled to bovine serum albumin by means of EDAC.

Method according to claim 3 wherein derivatives of petasin are used for immunization where the keto group in position 8 has been replaced by a carboxyl group and coupled to a bovine serum albumin through activated hydrazide dextran or fibrogen.

Method according to claims 4 and 5 wherein the insertion of the carboxyl group is

effected with carboxymethylhydroxyamine forming oxime.

Method according to claim 3 wherein derivatives of petasin are used for immunization where the double bond in positions 11, 12 is bromated and coupled to bovine serum albumin by means of a Traut's reagent.

Method according to claim 3 wherein derivatives of petasin are used for immunization where angelic acid is split off and the remaining petasol is coupled to a carrier through chloroformic acid ester.

Use of anti-petasin antibodies for detecting petasin or petasin protein conjugates in physiologic fluids.

Use according to claim 9 wherein they do not show any cross reactivity to derivatives, structural analogues or metabolites of petasin.

Use according to claims 9 and 10 wherein either petasin, petasin protein conjugates or anti-petasin antibodies are equipped with a marker.

Use according to claim 11 wherein markers are enzymes, fluorescent dyes, radio isotopes or redoxactive compounds.

Use according to one of the claims 9 to 12 wherein petasin bound to antibodies is detected optically, electrochemically, fluorimetrically or radiochemically.

Use according to claim 13 wherein a colour reagent is used.

Use according to claim 13 wherein the detection is carried out chromatographically.

Use according to one of the claims 9 to 15 wherein the reactants are present in a homologous solution.

Use according to one of the claims 9 to 16 wherein either anti-petasin antibodies, the petasin to be detected or the petasin protein conjugates are bound to a solid phase and a washing process takes place between the reaction steps.

Use according to claim 17 wherein anti-petasin antibodies, the petasin to be detected or the petasin protein conjugates are bound adsorptively to a solid phase or covalently after a preceding chemical activation of the solid phase.

Use according to claims 17 and 18 wherein the solid phase consists of polystyrene.

Use according to one of the claims 17 to 19 wherein the solid phase has a differing geometric shape.

Use according to claim 20 wherein in the form of a microtitration plate and a tube it

shows a spherical or planiform shape.

Test kit for detecting petasin in physiologic fluids comprising

anti-petasin antibodies,

a solid phase or polystyrene,

washing solution,

dilution buffer,

enzyme marked petasin.